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TITLE: MALARIA AND LEISHMANIASIS VECTOR ECOLOGY, TRANSMISSION, IMMUNOLOGY, PARASITOLOGY AND PROPHYLAXIS IN KENYA

PRINCIPAL INVESTIGATOR: Davy K. Koech, M.D., Ph.D.

CONTRACTING ORGANIZATION: Kenya Medical Research Institute
P.O. Box 54840
Nairobi, Kenya Africa 25427-2254

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Davy K. Koech, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Kenya Medical Research Institute
P.O. Box 54840
Nairobi, Kenya Africa 25427-2254

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13. ABSTRACT (Maximum 200 words)

Specific aims of the medical and biomedical research conducted in accordance with the statement of work for Cooperative Agreement DAMD17-92-V-2012 have involved two tropical diseases of Kenya, malaria and leishmaniasis. Being major health risks, both diseases possess significant relevance to military operations in tropical and subtropical areas of the world. The growing capability to identify specific parasite proteins and through reverse methods identify, clone and express their DNA fragments, has increasingly directed attention of Walter Reed Army Institute of Research (WRAIR) scientists toward immunologic studies for malaria vaccine development. Additionally, special emphasis is focused on identity, characterization, and determining the role of cytokines that are significant in immunity to malaria. In conjuction with these investigative efforts, arrangements are on-going to test two malaria vaccine candidates in Kenya. Studies of malaria vector ecology and transmission characterization is being accomplished to support the testing of the two and other malaria vaccine candidates. Production of serum-free medium for culture of cells and pathogenic

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protozoa have been developed and tested for production of parasite proteins free of exogenous serum and other reactogenic molecules.

Generation of information of leishmaniasis vector biology and parasitology with special attention directed toward studies relating to implementation of Polymerase chain reaction (PCR), DNA hybridization, and biochemical characterization techniques for detection and identification of Leishmania parasites predominate the leishmanial information acquired during the first half of this Cooperative Agreement. A new species of sandfly vector for leishmniasis was identified from the Baringo District, Kenya and essential information regarding immunodiagnostic/protective functions and characterization of an East African nonhuman primate (Vervet monkey) to act as a model for Leishmania vaccine development are advancing. Publication regarding these finding and scientific data are summarized or referenced in the text.

FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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Malaria and Leishmaniasis Vector Ecology, Transmission, Immunology, Parasitology and Prophylaxis in Kenya

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MID-TERM REPORT

KEMRI COOPERATIVE AGREEMENT DAMD17-92-V-2012

Malaria and Leishmaniasis Vector Ecology, Transmission, Immunology, Parasitology and Prophylaxis in Kenya

The Purpose of this mid-term report is to fulfill the requirements of Document No. DAMD17-92-V-2012 and to report in narrative summary the research findings of tropical diseases investigated. The aim is to summarize in manuscript format all studies funded and performed during the period 1 July 1992 through 15 December 1993.

Malaria:

INTRODUCTION:

Nature of The Problem: Malaria is a protozoal infection caused by members of the genus *Plasmodium*. Four species infect man -- *P. falciparum, P. vivax, P. malariae, and P. ovale.* The disease is widespread in most tropical areas of the world to include Kenya, may have a high attack rate, and high morbidity. It has been a major problem for humans throughout history and even today in endemic areas it continues to remarkably affect vitality and to cause death. As recent as a decade ago according to WHO estimates, approximately 96 million people in Africa alone had malaria. Although indigenous people acquire partial immunity, repeated infections cause periodic fever, indolence, fatigue and deaths in the young and old. Malaria is especially important to the armed forces when maneuvers and wars expose large numbers of susceptible troops to the disease. *Plasmodium falciparum* malaria is by far the most common variety accounting for 80-85% of reported cases in Kenya. Immunity has assumed the greatest role for controlling and preventing the malaria's, however much remains to be understood about the basic biochemical factors involved in host resistance to the disease. Also, there are problems complicated by strains of parasites which resist antimalarial drugs.

Background of Previous Work: Immune responses to malaria parasites has been induced in humans and other animals by injection of live, irradiated sporozoites. This immunity is mediated by immune cells and antibody with specificity by species and stage development of the malaria parasite. Dame et al.,(1990) reported the successful

cloning and sequencing of the gene that encodes the circumsporozoite (CS) protein of *P. falciparum*. Antibodies raised against synthetic peptides and recombinantly produced constructs from the unique repeating sequence of the *P. falciparum* CS protein have been shown to possess biologic activities possibly predictive of protection against sporozoite challenge, i.e., they mediate the circumsporozoite precipitation reaction and block sporozoite invasion of hepatocytes in vitro. These studies have led to development of vaccine candidates designed to protect against infection with the sporozoite stage of *P. falciparum* which has been tested in a Phase I safety and immunogenicity trial and a small phase IIa efficacy trial in volunteers in the United States. Although this initial vaccine candidate was not sufficiently immunogenic to justify phase IIb trials, additional vaccine candidates using improved adjuvants and specific proteins of the sporozoite and blood stages of the malaria parasite are under development by WRAIR and other investigators.

Kenya being chosen as a primary site for testing malaria vaccine candidates, directed many investigators to study and understand as much as possible the nature of the disease in endemic areas of the country. Research data now provide the information that will enable accurate estimation of the expected P. falciparum attack rate in volunteers staying in endemic areas for 2 to 8 weeks and longer and the optimal time to conduct vaccine trials. Data has been collected which enable investigators to confidently predict peak transmission periods. Entomologic and epidemiologic information is being advanced to provide factual data on situations such as rainfall, altitude, humidity and other endemic or seasonal factors.

Purpose and Approach of The Present Studies: The primary purpose of the proposed immunology, vector ecology and, transmission studies are to provide understanding, and the ultimate aim is to understand the mechanisms well enough to protect/prevent the disease. Studies to test the relationship between incidence of malaria and T lymphocyte responses to at least three malaria antigens: the circumsporozoite protein from sporozoites, the 70 kilodalton heatshock protein from liver stages, and the RESA antigen from blood stages. Conduct a retrospective case-control study of Kenyans previously identified as susceptible or resistant to malaria. Genetic factors such as HLA, G6PD, and pyridoxal kinase levels will be assessed for their contribution to malaria incidence. Continue to collect malaria parasites, sera and mononuclear cells for a storage bank for current and future studies of humoral and cellular immunity to malaria antigens. Study the epidemiology of falciparum malaria near Lake Victoria south of Kisumu in a location with annual periods of very transmission. Identify and characterize cytokines secreted in relation to *Plasmodium spp* infections and evaluate their role in immunity to malarial antigens in humans.

Malaria vector ecology and transmission studies are directed to refine estimates of malaria challenge in houses of volunteers, in support of evaluation of vaccine and immunology studies. Determine whether there is a relevant vector component in observed apparent "resistance" of some individuals to human malaria. Evaluate WRAIR-supplied repellents for the prevention of malaria. Implement histochemical assays for differentiating among species of human malaria's. Comparison of sensitivity and specificity with standard ELISA techniques. Implement and test specificity of non-radioactive methodologies for identification of vector members of the *Anopheles gambiae* complex. Study differential behavior of malaria vector species. Maintain baseline entomological data collection at a malaria vaccine trial site in western Kenya

Produce natural proteins from cells and organisms cultivated in protein-free media. These proteins are a potential source for vaccine and diagnostic, therapeutic, and research products.

Body: Summaries of the malaria research activity for the first half of the cooperative agreement efforts follows. Results obtained will be listed as publications and/or summarized in abstract format if not published.

A T cell Clone Which Protects Against both Plasmodium berghei and P. yoelii Sporozoites.

Weiss, W.R. et al., Journal of Immunology (1992).

The Role of CD4+ T cells in Protection Against Malaria Sporozoites.

Weiss, W.R. et al., Journal of Immunology (1993).

Malaria Vaccine Strategies, in "New Strategies for Parasitic Vaccines", International Laboratory for Research in Animal Diseases, Nairobi, Kenya

Weiss, W.R. et al., (in press) (1994).

Evaluation of a Blood Dipstick Test for Diagnosing Falciparum Malaria Infections.

Beadle, C., G. Long, W.R. Weiss, et al. Lancet (in press) (1994).

Completed field work in two protocols evaluating primaquine as a prophylactic drug against falciparum malaria as compared to standard drugs. Results show that daily primaquine is effective as mefloquine or doxycycline. This work has been presented at two national conferences, and will be submitted for publication in 1994.

In collaboration with LTC Sam Martin, USAMRU-Kenya, data show that genetic deficiencies of red cell pyridoxal kinase protect persons against malaria in Kenya. This effect is stronger than the previously known effect of G6PD deficiency.

Measured T cell responses in malaria in exposed volunteers against the malaria vaccine spf66 formulated at WRAIR. We find that 50% of persons tested in western Kenya have a vigorous proliferative T cell response. Also, Spf66 stimulates IL-4 production by T cells, but does not stimulate any detectable interferon-gamma production. This may explain why only a small percentage of adults are protected by this vaccine.

Measured T cell responses to segments of the P. falciparum circumsporozoite protein, a possible vaccine component, in persons naturally exposed to malaria. We find that naturally occurring CS variants are recognized by T cells of different persons, which may help in designing a multivalent malaria vaccine.

Discovered that persons with natural malaria resistance have stronger IL-4 responses to the IH3R epitope of the CS protein. This IL-4 comes from CD4+ T cells. This has implications for the type of vaccine constructs and adjuvants which should be tried with malaria vaccines.

Malaria in An Area of Marginal Rainfall along Lake Victoria, Western Kenya: Distribution of *Anopheles* Vectors and Marked Local Differences in *Plasmodium falciparum* Infection rates of *Anopheles gambiae*

Copeland, R.S., J. Chimumbwa, H. Ochieng, J.A. Onyona, M.O. Agawo, C. Asiago, J. Kamanza, J. Koros, M. Ouko, T. Whittle, and C.R. Roberts. (abstract) (1992).

Variation in Malaria Challenge Over Short Distances in a Holoendemic Area in Western Kenya: Implications for Vaccine Trials and Immunology Studies.

Copeland, R.S., F.K. Onyango, C. Oyaro, M. Omondi, C. Asiago, J. Kamanza, and W. Weiss. (Abstract) (1993)

Field Evidence of Differential Infectivity of Human Malaria Species to *Anopheles arabiensis* and *Anopheles gambiae*.

Copeland, R.S., J. Chimumbwa, J. Onyona, T. Whittle, C. Asiago, J. Kamanza, J. Koros, M. Ouko, and C.R. Roberts. (Abstract) (1993).

Response of Plasmodium falciparum Malaria to Chloroquine and Three Second Line Anti-malaria Drugs in Kenyan School-age Population.

Hagos, B., B. Khan, A.V. Ofulla, D. Kariuki, S.K. Martin. Am J Trop Med Hyg (1993).

Cultivation of Plasmodium falciparum in a Serum-free Medium.

Ofulla, A.V., V.C. Okoye, J.I. Githure, B. Khan, C.R. Roberts, A.J. Johnson, S.K. Martin. Am J Trop Med Hyg (1993)

Correlation of Phosphoinositide Hydrolysis with Exflagellation in the Malaria Microgemetocytes.

Martin, S.K., M. Jett, I. Schneider (in-press) (1993)

Factors Affecting Exflagellation of in vitro Cultivated Plasmodium falciparum Gametocytes.

Qwan'g, R.A., J.K. Mwangi, J.I. Githure, J.B. Were, C.R. Roberts, S.K. Martin. Am J Trop Med Hyg (1993)

Use of Pharmacological Agents to Implicate A Role for Phosphoinositide Hydrolysis Products in Malaria.

Owan'g, R.A., J.K. Mwangi, G. Gachihi, A Nwachukwu, C.R. Roberts, S.K. Martin. Biochem Pharmacol (1993)

Effects of Erythrocyte Pyridoxal Kinase Activity on The in vitro Growth Rates of Plasmodium falciparum

Inyama, J.S., A.S. Orago, A.V. Ofulla. N. Mulaya, A.A. Oloo, C.R. Roberts, S.K. Martrin. J Protozool Res (in-press) (1993)

Chloroquine-Resistant Plasmodium falciparum and The MDR-Phenotype.

Martin, S.K. Parasitol Today (1993)

Determination of Fifty Percent Inhibitory Concentration (IC50) of Anti-malarial Drugs Against Plasmodium falciparum Parasites in A Serum-free Medium.

Ofulla, A.V., A.S. Orago, J.L. Githure, J.P. Burans, G.M. Aleman, A.J. Johnson, and S.K. Martin. Am J Trop Med Hyg (in-press) (1993)

A One-step Technique For The Isolation, Transport and Short Term Storage of Plasmodium falciparum Parasites.

Ofulla, A.V., S.A. Orago, J.I. Githure, J.P. Burans, G.M. Aleman, A.J. Johnson, and S.K. Martin. Am J Trop Med Hyg (in-press) (1994)

Arachidonic Acid Metabolite in Cultivated Plasmodium falciparum.

Okoye, V.N., H.L. Williams, D.J. Johnson, S.K. Martin. Exper Parasitol (1993)

Leishmaniasis:

INTRODUCTION::

Nature of The Problem: Leishmaniasis is actually a complex group of diseases caused by parasites of the genus *Leishmania* and transmitted by sandflies of primarily the genus *Phlebotomus*. For visceral leishmaniasis (kala-azar), caused by *L. donovani*, the internal organs are primarily affected causing the patient to suffer irregular fever, anemia, weight loss, and enlargement of the spleen and liver. Identification of the parasite is the only reliable diagnostic test. In kala-azar examination of spleen or bone marrow puncture biopsy or less reliably by culturing peripheral blood on suitable culture media before examination are means for parasite identity. After a prepatent period of several months, without treatment, kala-azar can progress until death occurs usually after a few years. In cutaneous leishmaniasis, generally caused by *L. tropica* in Kenya, the disease usually remains localized in the skin. Cutaneous leishmaniasis diagnosis is enhanced by examining tissue or fluid from the edge of the skin lesion before or after culture in suitable media. Patients recovering from leishmaniasis usually acquire a lasting immunity to the particular parasite causing the disease. Kala-azar is a notifiable disease in Kenya. The epidemiology of the disease is mainly characterized by the habits of the sandfly which transmit the disease. In Kenya, sandflies are mainly found below 3,000 feet in hot, dry areas where the termite *Macrotermes sp.* builds its huge termite hills and it is in the ventilation shafts of such termite hills where the *Phlebotomus spp.* prefers to rest. As the flight range of the sandflies is limited, and generally do not move more than a few hundred meters form their resting sites, humans generally contract the disease in the neighborhood of termite hills. The Baringo, West Pokot, Turkana, Meru, and Machakos districts of Kenya fulfill the conditions for the termites and sandflies.

Background of Previous work: Historically, the primary research focus in leishmaniasis has been identification and characterization of the Leishmania parasite and defining the disease in the host. The classic detection and descriptive pathology has depended on a variety of factors: geographic location, clinical manifestations, vector and host specificities, behavior in in-vitro and in-vivo cultures, and serologic and immunologic assays. Although all of these parameters remain important in the diagnosis and characterization of Leishmania infections, they are insufficient for definitive parasite identification and disease diagnosis. Promastigotes and amastigotes of different Leishmania species and other related Kinetoplastida flagellates are usually morphologically indistinguishable in their respective stages when examine by ordinary light or electron microscopy, which is the most definitive means for identifying the parasite and detecting the disease. Such has resulted in a confused array of Leishmania categories and misidentifications. Emphasis for characterizing and identifying Leishmania by today's standards include specific enzyme/isoenzyme use

and DNA-based methods. While this biotechnology will remarkably enhance and improve our recognition and detection of leishmaniasis and leishmania parasites, much remain to be done to take full advantage of these techniques.

Purpose and Approach To The Present Studies: For the pass ten (10) years great research efforts has been focused on entomological-parasitological surveys for Leishmania spp. and leishmaniasis in different geographical areas of Kenya. It is well-known that human cutaneous leishmaniasis in the old world is caused by L. major, *L. tropica, L. aethiopica, and L. infantum.* A fifth leishmanial flagellate causing diffuse cutaneous leishmaniasis in Namibia and Tanzania awaits identification. Identification of a new rural focus of cutaneous leishmaniasis caused by L. tropica has occurred in Muruku of the Laikipai plateau, Kenya. *L. donovani,* which causes kala azar or visceral leishmaniasis and cutaneous lesions is also reported in the Baringo District, Rift Valley Province of Kenya. Efforts to delineate the distribution of cutaneous leishmaniasis through case-findings and vector and reservoir surveys continues. Recently, the sandfly Phlebotomus guggisbergi was found to be a vector of *L. tropica* transmission.

Additionally, leishmaniasis, both cutaneous and visceral forms, being endemic in Kenya has engendered research collaboration on the assessment of immunodiagnostic/protective functions and characterization of an East African nonhuman primate (Vervet monkey) to act as model for leishmania vaccine development and assessment of protective immunologic responses. Comparative immunological assessment of human and vervet L. major and/or L. tropica and L. donovani infections and cross-reactive studies to assess putative immunodiagnostic and protective antigens are to follow.

Body: Summaries of the Leishmania research activity for the first half of the cooperative agreement efforts follows. Results obtained will be listed as publications and/or summarized in abstract format if not published.

Biochemical characterization and zymodeme Classification of Leishmania Isolates From Patients, Vectors, and Reservoir Hosts in Kenya

Mebrahtu, Y.B., P.G. Lawyer, H. Pamba, D. Koech, P.V. Perkins, C.R. Roberts, JB Were, and L.D. Hendricks. Am. J. Trop. Med. Hyg., 47(6), 1992, pp. 852-892.

Identification of Phlebotomine Sandfly Bloodmeals From Baringo District, Kenya, By Direct Enzyme-linked Immunosorbent Assay (ELISA)

Ngumbi, P.M., P.G. Lawyer, R.N. Johnson, G. Kiilu, and C. Asiago. Medical and Veterinary Entomology, 1992, 6, pp. 385-388.

A New Rural Focus of Cutaneous Leishmaniasis Caused by Leishmania tropica in Kenya.

Mebrahtu, Y.B., P.G. Lawyer, P.M. Ngumbi, G. Kirigi, J. Mbugua, G. Gachihi, K. Wasunna, H. Pamba, J.A. Sherwood, D.K. Koech, and C.R. Roberts. Transactions of The Royal Society of Tropical Medicine an Hygiene, 1992, 86, pp. 381-387.

Leishmania donovani Parasites in The Nasal Secretions, Tonsillopharyngeal mucosa, and Urine Centrifugates of Visceral Leishmaniasis Patients in Kenya.

Mebrahtu, Y.B., L.D. Hendricks, C.N. Oster, P.G. Lawyer, P.V. Perkins, H. Pamba, D.K. Koech, and C.R. Roberts. American Journal of Tropical Medicine and Hygiene, 48(4), 1993, pp. 530-535.

Host Feeding Preference of Phlebotomus guggisbergi, A Vector of Leishmania tropica in Kenya.

Johnson, R.N., P.M. Ngumbi, J.P. Mwanyumba, and C.R. Roberts. Medical and Veterinary Entomology, 1993, 7, pp. 216-218.

Human Cutaneous Leishmaniasis Caused By Leishmania donovani s.l. in Kenya.

Mebrahtu, Y.B., G.V. Eys, I. Guizani, P.G. Lawyer, H. Pamba, D.K. Koech, C.R. Roberts, P.V. Perkins, J.B. Were, and L.D. Hendricks. Transactions of The Royal Society of Tropical Medicine and Hygiene, 1993, 87, pp. 598-601.

Phlebotomine Sandflies of Kenya (Diptera: Psychodidae). II. Phlebotomus aculeatus As A Probable Vector of Leishmania tropica s.l.

Johnson, R.N., R. Killick-Kendrick, and S.E.O. Meredith, Annals of Tropical Medicine and Parasitology, Vol. 87, No. 0,000-000, 1993.

Phlebotomine Sandflies Associated with Households of Human Visceral Leishmaniasis Cases in Baringo District, Kenya.

Robert, L.L., K.U. Schaefer, and R.N. Johnson, (In Press) Transaction of The Royal Society of Tropical Medicine and Hygiene.

Experimental Infection of Domestic Goats with Leishmania major Through Bites of Infected Phlebotomus duboscqi and Needle Inoculation of Culture-Derived promastigotes.

Anjili, C.O., J.O. Olobo, P.A. Mbati, L.L. Robert, and J.I. Githure, (Manuscript)

Experimental infection of four outbred domestic local Masai goats with Leishmania major were conducted. Two goats were inoculated intradermally in the right ear pinna with 1x10/7 stationary phase culture promastigotes mixed with Phlebotomus duboscqi salivary gland lysates. The other two goats were each subjected to bites of 11 L. major-infected P. duboscqi at similar sites. Both groups of goats developed small transient nodules that did not progress to ulceration. parasites were demonstrated at 28 and 42 days post-inoculation in the needle-inoculated group by culture of saline aspirates taken from the site of inoculation, but at 70 and 180 days post-inoculation, cultures did not reveal any parasites. Aspirate cultures taken from the sandfly bite group of goats did not reveal any parasites throughout the sampling period. Xenodiagnosis using P. duboscqi carried out on all goats at 56 and 70 days post-inoculation failed to reveal parasites. Throughout the study period, none of the goats in either group developed classical L. major lesions. It was concluded from these experiments that local domestic Masai goats could only be transiently infected with L. major promastigotes and are unlikely reservoirs nor secondary hosts for these parasites.

Parasitological and Serological Survey of Domestic Goats and Sheep For Leishmaniasis in Baringo District, Kenya (Abstract)

Robert, L.L., A.M. Shatry, C.O. Anjili, K.U. Schaefer, P.A. Mbati, and J.I. Githure (

Domestic goats have been suggested as reservoirs for leishmaniasis in Kenya and South Africa. Research performed at the Kenya Medical Research Institute demonstrated that domestic goats can act as transient reservoirs for Leishmania major for up to two months following needle inoculation with promastigotes or bites of infected sandflies. In an attempt to determine if goats act as natural reservoirs of leishmaniasis, we conducted a parasitological and serological study of goats and sheep in Baringo District, Kenya. A total of 102 goats and 7 sheep were sampled for the presence of natural infections with leishmania parasites at houses of recent human visceral leishmaniasis cases. Blood, lymph, bone marrow and sub-cutaneous samples were drawn from each animal and cultured in NNN media. No flagellates protozoans were cultured from the samples. However, the presence of Leishmania-specific antibodies were detected by ELISA in 7 goats (7.4%) and one sheep (14.3%). This indicates that goats and sheep can become infected with Leishmania parasites and produce a detectable antibody response. This research casts significant doubt upon the suggestion that goats and sheep can act as a reservoir for leishmaniasis and transmit the disease to humans in Kenya.